AGTCCCAGACGGGCTTTTCCCAGAGAGCTAAAAGAGAAGGGCCAGAGA**ATG**TCGTCCCAG
CCAGCAGGGAACCAGACCTCCCCCGGGGCCACAGAGGACTACTCCTATGGCAGCTGGTAC
ATCGATGAGCCCCAGGGGGGCGAGGAGCTCCAGCCAGAGGGGGAAGTGCCCTCCTGCCAC
ACCAGCATACCACCCGGCCTGTACCACGCCTGCCTGGCCTCGCTGTCAATCCTTGTGCTG
CTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTGACTGTGTGCGTGGCAGG
CCCGGCCTGCCCAGCCCTGTGGATTTCTTGGCTGGGGACAGGCCCCGGGCAGTGCCTGCT
GCTGTTTTCATGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTG
CCCTTCCTGACTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGG
GCCTGGAAGATACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGT
GCCACGGCTGGCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTT
GGGGTCCAGGTCTGGCAGAGGGCAGAGTGTCCCCAGGTGCCCAAGATCTACAAGTACTAC
TCCCTGCTGGCCTCCCTGCCTCTCCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCT
GTGCAGCTGGTGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGC
AGCTACTCTGAGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTAC
CACACCTCCAAGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTAC
ACTCCACAGCCAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGG
ACGGCCATTTACCAGGTGGCCCTGCTGCTGCTGGTGGGCGTGGTACCCACTATCCAGAAG
GTGAGGGCAGGGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTC
TCCGAGGACAAGCAGGAGGTGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTGGAAGTG
TGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCA
CTGGTGACACACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGT
CCCTTGCATCGGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGT
GCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCTTCCTG
GGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTG
CTCTTCCGTTCCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATC
CTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTG
ACCAACCGGCGAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTGCTGGTG
GGTGCCATGGTGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTT
GGCCAGATGGACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTAC
ACGTACCGAAACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTC
TGCTCCCTGCTCCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGAC
AGCCTCAGACCAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATG
GCCAAGGGAGCTAGGCCCGGGGCCAGCCGCGGCAGGGCTCGCTGGGGTCTGGCCTACACG
CTGCTGCACAACCCAACCCTGCAGGTCTTCCGCAAGACGGCCCTGTTGGGTGCCAATGGT
GCCCAGCCC**TGA**GGGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCC
TGCCTACCATCCTCCTCCCTCCCCGGCTCTCCTCCCAGCATCACACCAGCCATGCAGCCA
GCAGGTCCTCCGGATCACTGTGGTTGGGTGGAGGTCTGTCTGCACTGGGAGCCTCAGGAG
GGCTCTGCTCCACCCACTTGGCTATGGGAGAGCCAGCAGGGGTTCTGGAGAAAAAAACTG
GTGGGTTAGGGCCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTC
CCTACCCTGGCTCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACT
CCAGCCCAGCTCCACCTCAGCCTTGGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCT
CACCCCCTCAGCGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGTCCTCTGGC
CTGCAGGGCAGCCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGA
GAGCCAGATATTTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTC
CCTGCAATAAACTTGTTCCTGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIG._1

Inventor: Pennica et al.
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```
MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASLS
ILVLLLLAMLVRRRQLWPDCVRGRPGLPSPVDFLAGDRPRAVPAAVFMVLLSSLCLLLPD
EDALPFLTLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLS
WAHLGVQVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSK
GLQSSYSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSA
TLTGTAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLW
ALEVCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCW
MSFSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLA
LAVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAMVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTMA
APQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTALL
GANGAQP
```

**Important features of the protein:**
**Signal peptide:**

**None**

**Transmembrane domain:**

54-69
102-119
148-166
207-222
301-320
364-380
431-451
474-489
560-535

Motif file:
Motif name: N-glycosylation site.

8-12

Motif name: N-myristoylation site.

50-56
176-182
241-247
317-323
341-347
525-531
627-633
631-637
640-646
661-667

Motif name: Prokaryotic membrane lipoprotein lipid attachment site.

364-375

Motif name: ATP/GTP-binding site motif A (P-loop).

132-140

**FIG._2**

```
PRO                      XXXXXXXXXXXXXXX      (Length = 15 amino acids)
Comparison Protein       XXXXXYYYYYYY         (Length = 12 amino acids)
```

% amino acid sequence identity =
(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
5 divided by 15 = 33.3%

FIG._3A

```
PRO                      XXXXXXXXXX           (Length = 10 amino acids)
Comparison Protein       XXXXXYYYYYYZZYZ      (Length = 15 amino acids)
```

% amino acid sequence identity =
(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
5 divided by 10 = 50%

FIG._3B

```
PRO-DNA                  NNNNNNNNNNNNNN       (Length = 14 nucleotides)
Comparison DNA           NNNNNNLLLLLLLLLL     (Length = 16 nucleotides)
```

% nucleic acid sequence identity =
(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =
6 divided by 14 = 42.9%

FIG._3C

```
PRO-DNA                  NNNNNNNNNNNN         (Length = 12 nucleotides)
Comparison DNA           NNNNLLLVV            (Length = 9 nucleotides)
```

% nucleic acid sequence identity =
(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =
4 divided by 12 = 33.3%

FIG._3D

Inventor: Pennica et al.
Docket No.: 11669.0163USU1
Title: NOVEL STRA6 POLYPEPTIDES
Serial No.: 09/759,056
Sheet 4 of 35

FIG._4A

```
/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
#define _M   -8      /* value of a match with a stop */
int         _day[26][26] = {
/*     A B C D E F G H I J K L M N O P Q R S T U V W X Y Z*/
/* A */    { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0,_M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
/* B */    { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2,_M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
/* C */    {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4,_M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
/* D */    { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
/* E */    { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
/* F */    {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4,_M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
/* G */    { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0,_M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
/* H */    {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2,_M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
/* I */    {-1,-2,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2,_M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
/* J */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */    {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1,_M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
/* L */    {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */    {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
/* N */    { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2,_M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
/* O */    {_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,0,_M,_M,_M,_M,
                 _M,_M,_M,_M,_M,_M,_M},
/* P */    { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1,_M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
/* Q */    { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1,_M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
/* R */    {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0,_M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
/* S */    { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1,_M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
/* T */    { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0,_M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
/* U */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */    { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
/* W */    {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
/* X */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */    {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
/* Z */    { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
};
```

FIG._4B

```
/*
 */
#include <stdio.h>
#include <ctype.h>

#define     MAXJMP     16     /* max jumps in a diag */
#define     MAXGAP     24     /* don't continue to penalize gaps larger than this */
#define     JMPS       1024   /* max jmps in an path */
#define     MX         4      /* save if there's at least MX-1 bases since last jmp */

#define     DMAT       3      /* value of matching bases */
#define     DMIS       0      /* penalty for mismatched bases */
#define     DINS0      8      /* penalty for a gap */
#define     DINS1      1      /* penalty per base */
#define     PINS0      8      /* penalty for a gap */
#define     PINS1      4      /* penalty per residue */

struct jmp {
            short              n[MAXJMP];       /* size of jmp (neg for dely) */
            unsigned short     x[MAXJMP];       /* base no. of jmp in seq x */
};                                              /* limits seq to 2^16 -1 */

struct diag {
            int        score;      /* score at last jmp */
            long       offset;     /* offset of prev block */
            short      ijmp;       /* current jmp index */
            struct     jmpjp;      /* list of jmps */
};

struct path {
            int        spc;        /* number of leading spaces */
            short      n[JMPS];    /* size of jmp (gap) */
            int        x[JMPS];    /* loc of jmp (last elem before gap) */
};

char        *ofile;                /* output file name */
char        *namex[2];             /* seq names: getseqs() */
char        *prog;                 /* prog name for err msgs */
char        *seqx[2];              /* seqs: getseqs() */
int         dmax;                  /* best diag: nw() */
int         dmax0;                 /* final diag */
int         dna;                   /* set if dna: main() */
int         endgaps;               /* set if penalizing end gaps */
int         gapx, gapy;            /* total gaps in seqs */
int         len0, len1;            /* seq lens */
int         ngapx, ngapy;          /* total size of gaps */
int         smax;                  /* max score: nw() */
int         *xbm;                  /* bitmap for matching */
long        offset;                /* current offset in jmp file */
struct diag *dx;                   /* holds diagonals */
struct path pp[2];                 /* holds path for seqs */

char        *calloc(), *malloc(), *index(), *strcpy();
char        *getseq(), *g_calloc();
```

FIG._4C

```
/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
 *   where file1 and file2 are two dna or two protein sequences.
 *   The sequences can be in upper- or lower-case an may contain ambiguity
 *   Any lines beginning with ';', '>' or '<' are ignored
 *   Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *   Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
#include "nw.h"
#include "day.h"

static _dbval[26] = {
        1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static _pbval[26] = {
        1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
        128, 256, 0xFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
        1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
        1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)                                                              main
        int     ac;
        char    *av[];
{
        prog = av[0];
        if (ac != 3) {
                fprintf(stderr,"usage: %s file1 file2\n", prog);
                fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                fprintf(stderr,"Any lines beginning with ';' or '<' are ignored\n");
                fprintf(stderr,"Output is in the file \"align.out\"\n");
                exit(1);
        }
        namex[0] = av[1];
        namex[1] = av[2];
        seqx[0] = getseq(namex[0], &len0);
        seqx[1] = getseq(namex[1], &len1);
        xbm = (dna)? _dbval : _pbval;

        endgaps = 0;            /* 1 to penalize endgaps */
        ofile = "align.out";    /* output file */

        nw();           /* fill in the matrix, get the possible jmps */
        readjmps();     /* get the actual jmps */
        print();        /* print stats, alignment */

        cleanup(0);     /* unlink any tmp files */
}
```

FIG._4D

```
/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()                                                              nw
{
        char        *px, *py;        /* seqs and ptrs */
        int         *ndely, *dely;   /* keep track of dely */
        int         ndelx, delx;     /* keep track of delx */
        int         *tmp;            /* for swapping row0, row1 */
        int         mis;             /* score for each type */
        int         ins0, ins1;      /* insertion penalties */
        register    id;              /* diagonal index */
        register    ij;              /* jmp index */
        register    *col0, *col1;    /* score for curr, last row */
        register    xx, yy;          /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;    /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
         */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                /* initialize first entry in col
                 */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
                else {
                        col1[0] = 0;
                        delx = -ins0;
                        ndelx = 0;
                }
```

```
for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
        mis = col0[yy-1];
        if (dna)
                mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
        else
                mis += _day[*px-'A'][*py-'A'];

        /* update penalty for del in x seq;
         * favor new del over ongong del
         * ignore MAXGAP if weighting endgaps
         */
        if (endgaps || ndely[yy] < MAXGAP) {
                if (col0[yy] - ins0 >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else {
                        dely[yy] -= ins1;
                        ndely[yy]++;
                }
        } else {
                if (col0[yy] - (ins0+ins1) >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else
                        ndely[yy]++;
        }

        /* update penalty for del in y seq;
         * favor new del over ongong del
         */
        if (endgaps || ndelx < MAXGAP) {
                if (col1[yy-1] - ins0 >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else {
                        delx -= ins1;
                        ndelx++;
                }
        } else {
                if (col1[yy-1] - (ins0+ins1) >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else
                        ndelx++;
        }

        /* pick the maximum score; we're favoring
         * mis over any del and delx over dely
         */
```

FIG._4E

...nw

```
id = xx - yy + len1 - 1;
if (mis >= delx && mis >= dely[yy])
        col1[yy] = mis;
else if (delx >= dely[yy]) {
        col1[yy] = delx;
        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                dx[id].ijmp++;
                if (++ij >= MAXJMP) {
                        writejmps(id);
                        ij = dx[id].ijmp = 0;
                        dx[id].offset = offset;
                        offset += sizeof(struct jmp) + sizeof(offset);
                }
        }
        dx[id].jp.n[ij] = ndelx;
        dx[id].jp.x[ij] = xx;
        dx[id].score = delx;
}
else {
        col1[yy] = dely[yy];
        ij = dx[id].ijmp;

if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                dx[id].ijmp++;
                if (++ij >= MAXJMP) {
                        writejmps(id);
                        ij = dx[id].ijmp = 0;
                        dx[id].offset = offset;
                        offset += sizeof(struct jmp) + sizeof(offset);
                }
        }
        dx[id].jp.n[ij] = -ndely[yy];
        dx[id].jp.x[ij] = xx;
        dx[id].score = dely[yy];
}
if (xx == len0 && yy < len1) {
        /* last col
         */
        if (endgaps)
                col1[yy] -= ins0+ins1*(len1-yy);
        if (col1[yy] > smax) {
                smax = col1[yy];
                dmax = id;
```

FIG._4F-1

```
                                }
                        }
                }
                if (endgaps && xx < len0)
                        col1[yy-1] -= ins0+ins1*(len0-xx);
                if (col1[yy-1] > smax) {
                        smax = col1[yy-1];
                        dmax = id;
                }
                tmp = col0; col0 = col1; col1 = tmp;
        }
        (void) free((char *)ndely);
        (void) free((char *)dely);
        (void) free((char *)col0);
        (void) free((char *)col1);
}
```

FIG._4F-2

FIG._4G

```
/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() - -put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC      3
#define P_LINE   256   /* maximum output line */
#define P_SPC    3     /* space between name or num and seq */

extern   _day[26][26];
int      olen;         /* set output line length */
FILE     *fx;          /* output file */

print()                                                         print
{
        int     lx, ly, firstgap, lastgap;      /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
                fprintf(stderr,"%s: can't write %s\n", prog, ofile);
                cleanup(1);
        }
        fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) {          /* leading gap in x */
                pp[0].spc = firstgap = len1 - dmax - 1;
                ly -= pp[0].spc;
        }
        else if (dmax > len1 - 1) {  /* leading gap in y */
                pp[1].spc = firstgap = dmax - (len1 - 1);
                lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) {         /* trailing gap in x */
                lastgap = len0 - dmax0 -1;
                lx -= lastgap;
        }
        else if (dmax0 > len0 - 1) {/* trailing gap in y */
                lastgap = dmax0 - (len0 - 1);
                ly -= lastgap;
        }
        getmat(lx, ly, firstgap, lastgap);
        pr_align();
}
```

FIG._4H

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                          getmat
        int     lx, ly;                         /* "core" (minus endgaps) */
        int     firstgap, lastgap;              /* leading trailing overlap */
{
        int                     nm, i0, i1, siz0, siz1;
        char                    outx[32];
        double                  pct;
        register                n0, n1;
        register char           *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
        pct = 100.*(double)nm/(double)lx;
        fprintf(fx, "\n");
        fprintf(fx, "<%d match%s in an overlap of %d: %.2f percent similarity\n",
                nm, (nm == 1)? "" : "es", lx, pct);
        fprintf(fx, "<gaps in first sequence: %d", gapx);                    ...getmat
        if (gapx) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapx, (dna)? "base":"residue", (ngapx == 1)? "":"s");
                fprintf(fx,"%s", outx);

        fprintf(fx, ", gaps in second sequence: %d", gapy);
        if (gapy) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapy, (dna)? "base":"residue", (ngapy == 1)? "":"s");
                fprintf(fx,"%s", outx);
        }
        if (dna)
                fprintf(fx,
                "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per
                 base)\n", smax, DMAT, DMIS, DINS0, DINS1);
        else
                fprintf(fx,
                "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per
                 residue)\n", smax, PINS0, PINS1);
        if (endgaps)
                fprintf(fx,
                "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
                firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
        else
                fprintf(fx, "<endgaps not penalized\n");
}

static          nm;                     /* matches in core -- for checking */
static          lmax;                   /* lengths of stripped file names */
static          ij[2];                  /* jmp index for a path */
static          nc[2];                  /* number at start of current line */
static          ni[2];                  /* current elem number -- for gapping */
static          siz[2];
static char     *ps[2];                 /* ptr to current element */
static char     *po[2];                 /* ptr to next output char slot */
static char     out[2][P_LINE];         /* output line */
static char     star[P_LINE];           /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
pr_align()                                                                   pr_align
```

FIG._4I-1

```
{
        int             nn;     /* char count */
        int             more;
        register                i;

        for (i =  0, lmax = 0; i < 2; i++) {
                nn = stripname(namex[i]);
                if (nn > lmax)
                        lmax = nn;

                nc[i] = 1;
                ni[i] = 1;
                siz[i] = ij[i] = 0;
                ps[i] = seqx[i];
                po[i] = out[i];
```

*FIG._4I-2*

**...pr_align**

```
        for (nn = nm = 0, more = 1; more; ) {
                for (i = more = 0; i < 2; i++) {
                        /*
                         * do we have more of this sequence?
                         */
                        if (!*ps[i])
                                continue;

                        more++;

                        if (pp[i].spc) {          /* leading space */
                                *po[i]++ = ' ';
                                pp[i].spc--;
                        }
                        else if (siz[i]) {        /* in a gap */
                                *po[i]++ = '-';
                                siz[i]--;
                        }
                        else {            /* we're putting a seq element
                                           */
                                *po[i] = *ps[i];
                                if (islower(*ps[i]))
                                        *ps[i] = toupper(*ps[i]);
                                po[i]++;
                                ps[i]++;

                                /*
                                 * are we at next gap for this seq?
                                 */
                                if (ni[i] == pp[i].x[ij[i]]) {
                                        /*
                                         * we need to merge all gaps
                                         * at this location
                                         */
                                        siz[i] = pp[i].n[ij[i]++];
                                        while (ni[i] == pp[i].x[ij[i]])
                                                siz[i] += pp[i].n[ij[i]++];
                                }
                                ni[i]++;
                        }
                }
                if (++nn == olen || !more && nn) {
                        dumpblock();
                        for (i = 0; i < 2; i++)
                                po[i] = out[i];
                        nn = 0;
                }
        }
}
/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()
{
        register        i;

        for (i = 0; i < 2; i++)
                *po[i]-- = '\0';
```

**dumpblock**

FIG._4J

...dumpblock

```
        (void) putc('\n', fx);
        for (i = 0; i < 2; i++) {
                if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                        if (i == 0)
                                nums(i);
                        if (i == 0 && *out[1])
                                stars();
                        putline(i);
                        if (i == 0 && *out[1])
                                fprintf(fx, star);
                        if (i == 1)
                                nums(i);
                }
        }
}

/*
 * put out a number line: dumpblock()
 */
static
nums(ix)                                                                nums
        int     ix;     /* index in out[] holding seq line */
{
        char            nline[P_LINE];
        register                i, j;
        register char           *pn, *px, *py;

        for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
                *pn = ' ';
        for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                if (*py == ' ' || *py == '-')
                        *pn = ' ';
                else {
                        if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                                j = (i < 0)? -i : i;
                                for (px = pn; j; j /= 10, px--)
                                        *px = j%10 + '0';
                                if (i < 0)
                                        *px = '-';
                        }
                        else
                                *pn = ' ';
                        i++;
                }
        }
        *pn = '\0';
        nc[ix] = i;
        for (pn = nline; *pn; pn++)
                (void) putc(*pn, fx);
        (void) putc('\n', fx);
}

/*
 * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
putline(ix)                                                             putline
        int     ix;
{
```

FIG._4K

...putline

```
        int                 i;
        register char       *px;

        for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * ni[] is current element (from 1)
         * nc[] is number at start of current line
         */
        for (px = out[ix]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}

/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
{
        int                 i;
        register char       *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
            !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {

                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                cx = '.';
                        else
                                cx = ' ';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
        *px = '\0';
}
```

stars

FIG._4L

```
/*
 * strip path or prefix from pn, return len: pr_align()
 */
static
stripname(pn)                                                   stripname
        char    *pn;    /* file name (may be path) */
{
        register char           *px, *py;

        py = 0;
        for (px = pn; *px; px++)
                if (*px == '/')
                        py = px + 1;
        if (py)
                (void) strcpy(pn, py);
        return(strlen(pn));

}
```

FIG._4M

```
/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjmps() -- get the good jmps, from tmp file if necessary
 * writejmps() -- write a filled array of jmps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>

char    *jname = "/tmp/homgXXXXXX";          /* tmp file for jmps */
FILE    *fj;

int     cleanup();                           /* cleanup tmp file */
long    lseek();

/*
 * remove any tmp file if we blow
 */
cleanup(i)                                                              cleanup
        int     i;
{
        if (fj)
                (void) unlink(jname);
        exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                                                       getseq
        char                    *file;  /* file name */
        int                     *len;   /* seq len */
{
        char                    line[1024], *pseq;
        register char           *px, *py;
        int                     natgc, tlen;
        FILE                    *fp;

        if ((fp = fopen(file,"r")) == 0) {
                fprintf(stderr,"%s: can't read %s\n", prog, file);
                exit(1);
        }
        tlen = natgc = 0;
        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++)
                        if (isupper(*px) || islower(*px))
                                tlen++;
        }
        if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
                fprintf(stderr,"%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6,
                file);
                exit(1);
        }
        pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

FIG._4N

```
        py = pseq + 4;
        *len = tlen;
        rewind(fp);

        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++) {
                        if (isupper(*px))
                                *py++ = *px;
                        else if (islower(*px))
                                *py++ = toupper(*px);
                        if (index("ATGCU",*(py-1)))
                                natgc++;
                }
        }
        *py++ = '\0';
        *py = '\0';
        (void) fclose(fp);
        dna = natgc > (tlen/3);
        return(pseq+4);
}

char    *
g_calloc(msg, nx, sz)                                                   g_calloc
        char    *msg;           /* program, calling routine */
        int     nx, sz;         /* number and size of elements */
{
        char            *px, *calloc();

        if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                if (*msg) {
                        fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg,
                         nx, sz);
                        exit(1);
                }
        }
        return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
readjmps()                                                              readjmps
{
        int             fd = -1;
        int             siz, i0, i1;
        register        i, j, xx;

        if (fj) {
                (void) fclose(fj);
                if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                        fprintf(stderr, "%s: can't open() %s\n", prog, jname);
                        cleanup(1);
                }
        }
        for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                while (1) {
                        for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                                ;
```

FIG._4O

```
                    if (j < 0 && dx[dmax].offset && fj) {
                            (void) lseek(fd, dx[dmax].offset, 0);
                            (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
                            (void) read(fd, (char *)&dx[dmax].offset,
                            sizeof(dx[dmax].offset));
                            dx[dmax].ijmp = MAXJMP-1;
                    }
                    else
                            break;
            }
            if (i >= JMPS) {
                    fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                    cleanup(1);
            }
            if (j >= 0) {
                    siz = dx[dmax].jp.n[j];
                    xx = dx[dmax].jp.x[j];
                    dmax += siz;
                    if (siz < 0) {          /* gap in second seq */
                            pp[1].n[i1] = -siz;
                            xx += siz;
                            /* id = xx - yy + len1 - 1
                             */
                            pp[1].x[i1] = xx - dmax + len1 - 1;
                            gapy++;
                            ngapy -= siz;
/* ignore MAXGAP when doing endgaps */
                            siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
                            i1++;
                    }
                    else if (siz > 0) {     /* gap in first seq */
                            pp[0].n[i0] = siz;
                            pp[0].x[i0] = xx;
                            gapx++;
                            ngapx += siz;
/* ignore MAXGAP when doing endgaps */
                            siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                            i0++;
                    }
            }
            else
                    break;
    }
```

FIG._4P-1

```
        /* reverse the order of jmps
         */
        for (j = 0, i0--; j < i0; j++, i0--) {
                i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
                i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
        }
        for (j = 0, i1--; j < i1; j++, i1--) {
                i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
                i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
        }
        if (fd >= 0)
                (void) close(fd);
        if (fj) {
                (void) unlink(jname);
                fj = 0;
                offset = 0;
        }}
```

FIG._4P-2

```
/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejmps(ix)                                                    writejmps
        int     ix;
{
        char    *mktemp();

        if (!fj) {
                if (mktemp(jname) < 0) {
                        fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
                        cleanup(1);
                }
                if ((fj = fopen(jname, "w")) == 0) {
                        fprintf(stderr, "%s: can't write %s\n", prog, jname);
                        exit(1);
                }
        }
        (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
        (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}
```

FIG._4Q

```
GTGCTCTCCGAGGACAAGCAGGAGGNGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTG
GAAGTGTGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATG
CGCTCACTGGTGACACACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGAC
TTGAGTCCCTTGCATCGGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGC
TTCAGTGCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTC
TTCCTGGGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAAC
CTCCTGCTCTTCCGTTCCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCT
GTGATCCTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCA
CAGCTGACCAACCGGCGAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTG
CTGGTGGGTGCCATGGTGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATC
CACCTTGGCCAGATGGACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGC
TACTACACGTACCGAA
```

FIG._5

CACAACCAGCCACCCCTCTAGGATCCCAGCCCAGCTGGTGCTGGGCTCAGAGGAGAAGGC
CCCGTGTTGGGAGCACCCTGCTTGCCTGGAGGGACAAGTTTCCGGGAGAGATCAATAAAG
GAAAGGAAAGAGACAAGGAAGGGAGAGGTCAGGAGAGCGCTTGATTGGAGGAGAAGGGCC
AGAGA**ATG**TCGTCCCAGCCAGCAGGGAACCAGACCTCCCCCGGGGCCACAGAGGACTACT
CCTATGGCAGCTGGTACATCGATGAGCCCCAGGGGGGCGAGGAGCTCCAGCCAGAGGGGG
AAGTGCCCTCCTGCCACACCAGCATACCACCCGGCCTGTACCACGCCTGCCTGGCCTCGC
TGTCAATCCTTGTGCTGCTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTG
ACTGTGTGCGTGGCAGGCCCGGCCTGCCCAGGCCCCGGGCAGTGCCTGCTGCTGTTTTCA
TGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTGCCCTTCCTGA
CTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGGGCCTGGAAGA
TACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGTGCCACGGCTG
GCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTTGGGGTCCAGG
TCTGGCAGAGGGCAGAGTGTCCCCAGGTGCCCAAGATCTACAAGTACTACTCCCTGCTGG
CCTCCCTGCCTCTCCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCTGTGCAGCTGG
TGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGCAGCTACTCTG
AGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTACCACACCTCCA
AGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTACACTCCACAGC
CAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGGACGGCCATTT
ACCAGGTGGCCCTGCTGCTGCTGGTGGGCGTGGTACCCACTATCCAGAAGGTGAGGGCAG
GGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTCTCCGAGGACA
AGCAGGAGGTGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTGGAAGTGTGCTACATCT
CAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCACTGGTGACAC
ACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGTCCCTTGCATC
GGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGTGCCTACCAGA
CAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCTTCCTGGGAACCACGG
CCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTGCTCTTCCGTT
CCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATCCTGCAGAACA
TGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTGACCAACCGGC
GAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTGCTGGTGGGTGCCATAG
TGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTTGGCCAGATGG
ACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTACACGTACCGAA
ACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTCTGCTCCCTGC
TCCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGACAGCCTCAGAC
CAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATGGCCAAGGGAG
CTAGGCCCGGGGCCAGCCGCGGCAGGGCTCGCTGGGGTCTGGCCTACACGCTGCTGCACA
ACCCAACCCTGCAGGTCTTCCGCAAGACGGCCCTGTTGGGTGCCAATGGTGCCCAGCCC**T**
**GA**GGGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCCTGCCTACCAC
CTCCTCCCTCCCCGGCTCTCCTCCCAGCATCACACCAGCCATGCAGCCAGCAGGTCCTCC
GGATCACTGTGGTTGGGTGGAGGTCTGTCTGCACTGGGAGCCTCAGGAGGGCTCTGCTCC
ACCCACTTGGCTATGGGAGAGCCAGCAGGGGTTCTGGAGAAAGAAACTGGTGGGTTAGGG
CCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTCCCTACCCTGGC
TCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACTCCAGCCCAGCT
CCACCTCAGCCTTGGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCTCACCCCCTCAG
CGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGGCCTCTGGCCTGCAGGGCAG
CCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGAGAGCCAGATAT
TTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTCCCTGCAATAAA
CTTGTTCCTGAGAAAAA

*FIG._6*

```
MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASL
SILVLLLLAMLVRRRQLWPDCVRGRPGLPRPRAVPAAVFMVLLSSLCLLLPDEDALPFL
TLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLSWAHLGV
QVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSKGLQSS
YSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSATLTG
TAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLWALE
VCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCWMS
FSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLAL
AVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAIVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTM
AAPQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTA
LLGANGAQP
```

**Important features of the protein:**
**Signal peptide:**
**none**

**Transmembrane domain:**

54-71
93-111
140-157
197-214
291-312
356-371
425-444
464-481
505-522

Motif name: N-glycosylation site.

8-12

Motif name: N-myristoylation site.

50-56
167-173
232-238
308-314
332-338
516-522
618-624
622-628
631-637
652-658

Motif name: Prokaryotic membrane lipoprotein lipid attachment site.

355-366

Motif name: ATP/GTP-binding site motif A (P-loop).

123-131

***FIG._7***

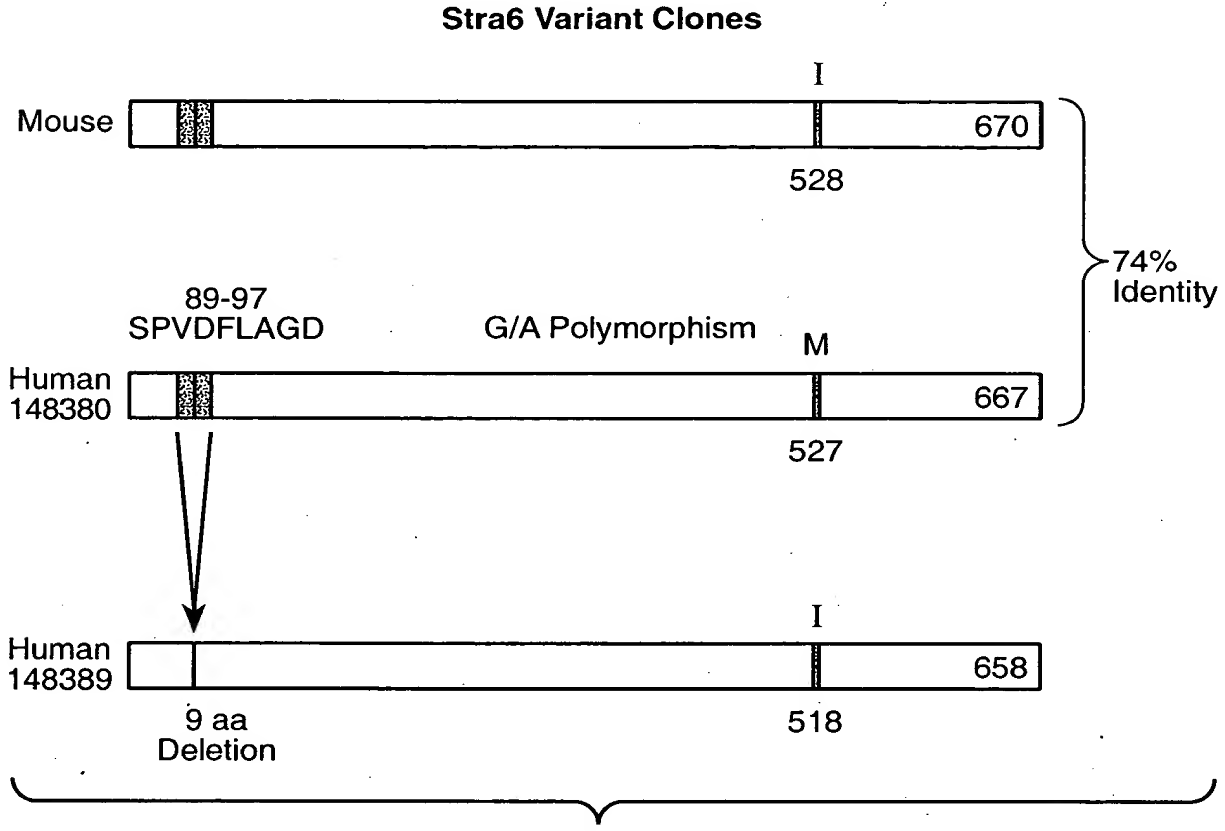
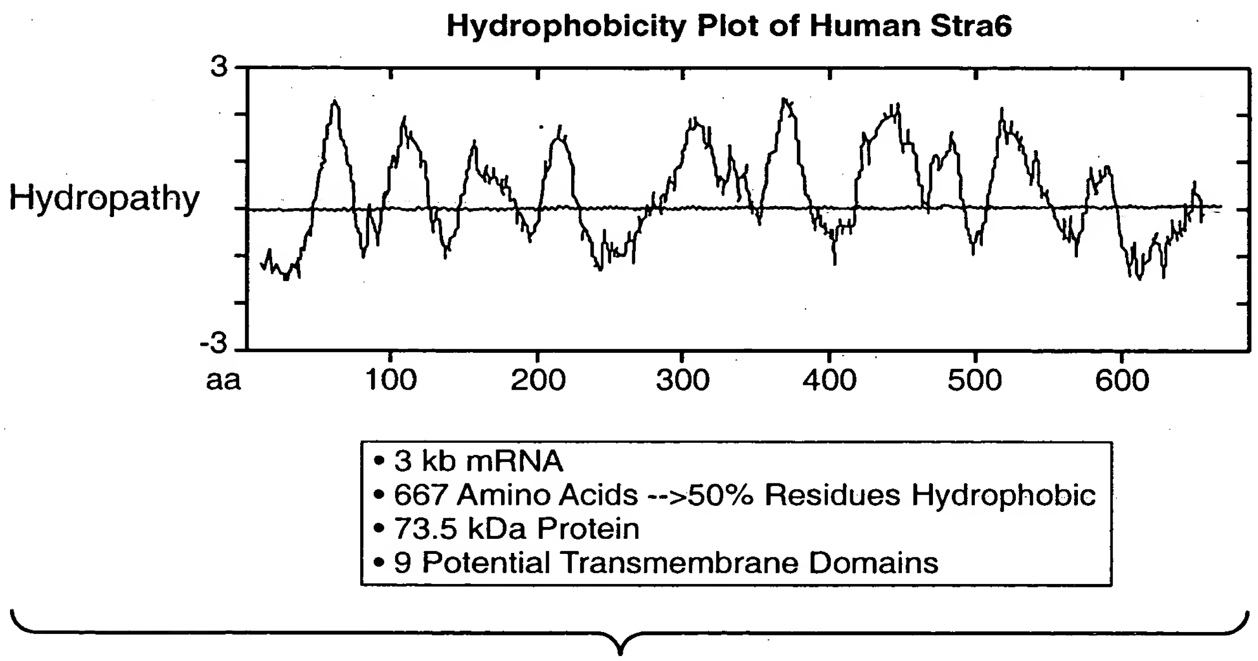


FIG._8



• 3 kb mRNA
• 667 Amino Acids -->50% Residues Hydrophobic
• 73.5 kDa Protein
• 9 Potential Transmembrane Domains

FIG._9

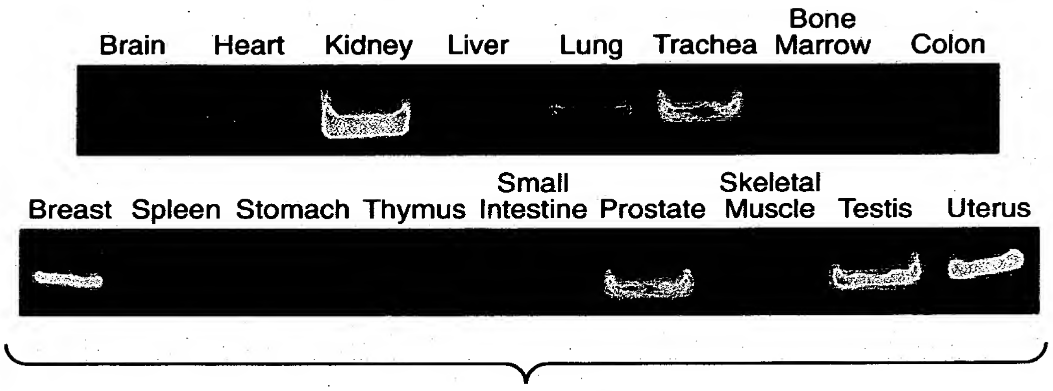

Brain
Heart
Kidney
Liver
Lung
Trachea
Bone Marrow
Colon
Breast
Spleen
Stomach
Thymus
Small Intestine
Prostate
Skeletal Muscle
Testis
Uterus

FIG._10

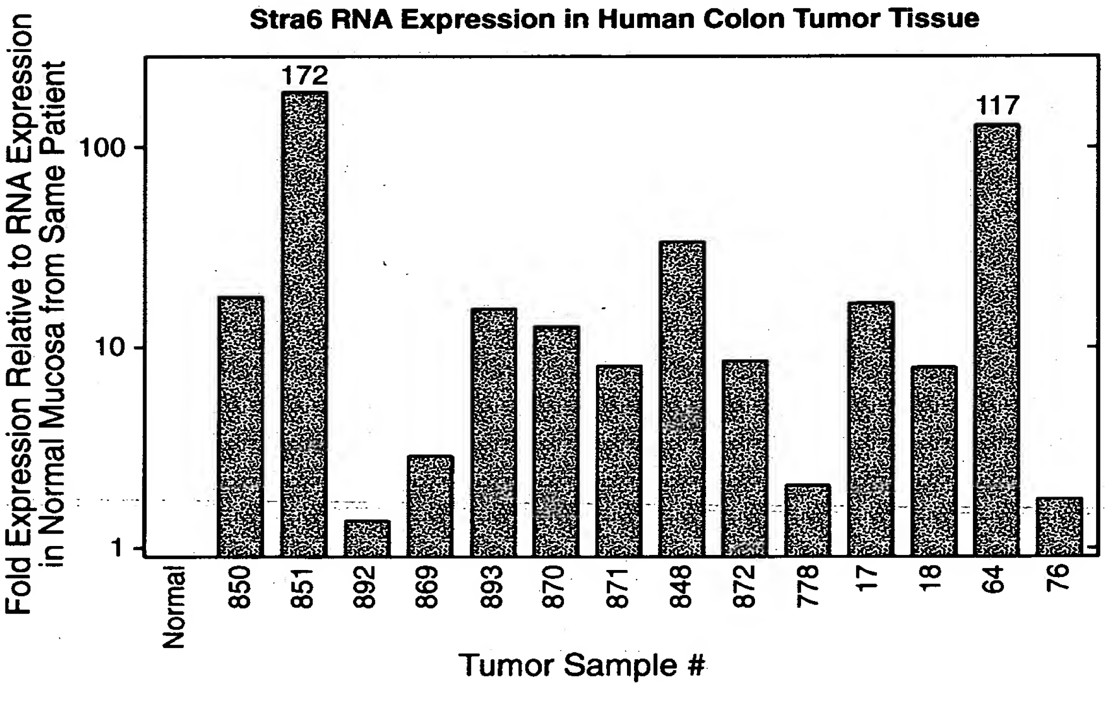

Stra6 RNA Expression in Human Colon Tumor Tissue
Fold Expression Relative to RNA Expression in Normal Mucosa from Same Patient
172
117
100
10
1
Normal
850
851
892
869
893
870
871
848
872
778
17
18
64
76
Tumor Sample #

FIG._11

Inventor: Pennica et al.
Docket No.: 11669.0163USU1
Title: NOVEL STRA6 POLYPEPTIDES
Serial No.: 09/759,056
Sheet 28 of 35

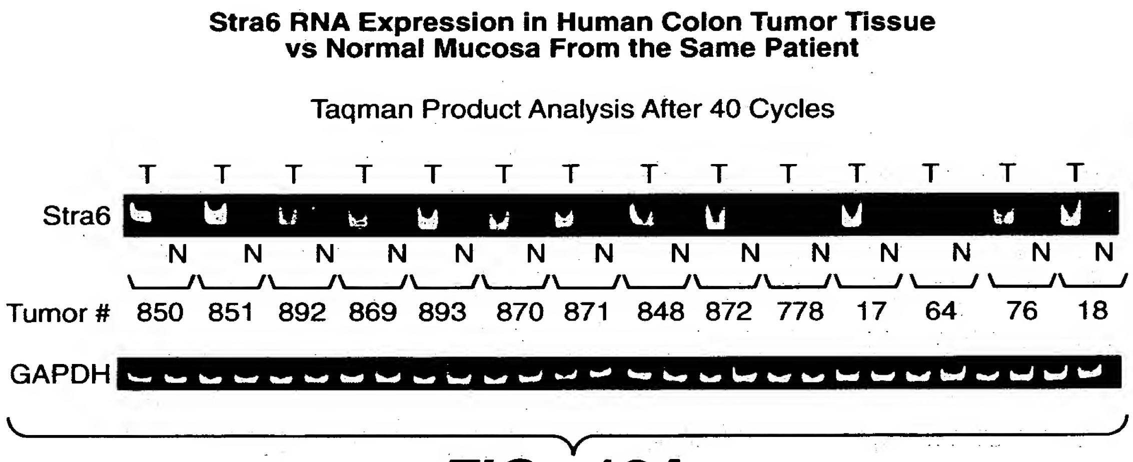


FIG._12A

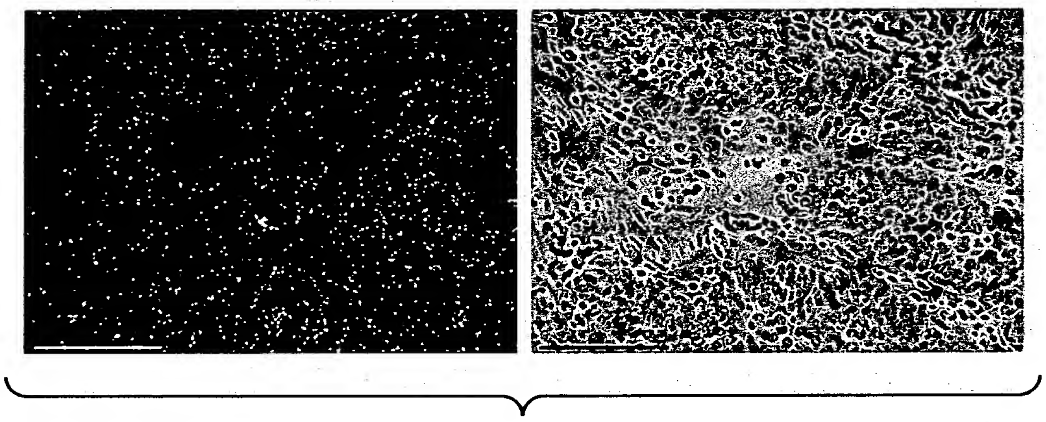


FIG._12B

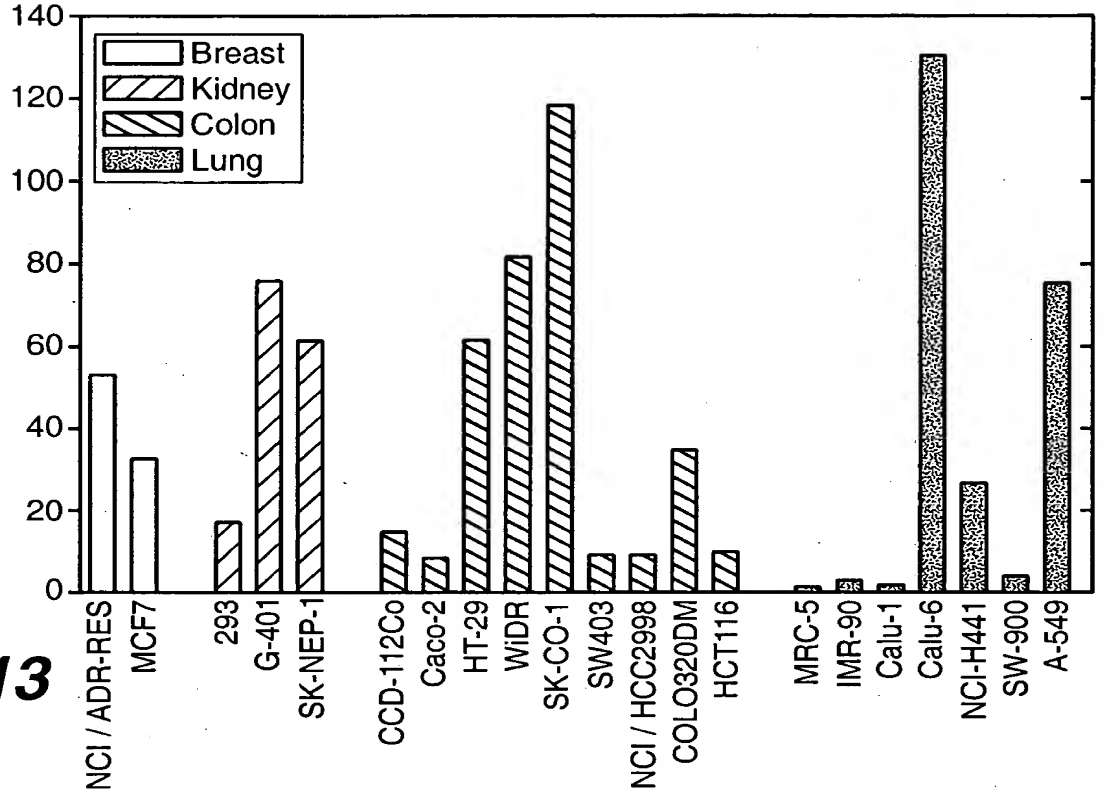


*FIG._13*

Stra6 RNA Expression in Human Colon Carcinoma Cells + / - Retinoic Acid

TM #75 (2/28/00) VD3 - Vitamin D3 (1μM); ATRA - All-Trans-Retinoic Acid (1μM); 9cRA - 9-Cis-Retinoic Acid (1μM)

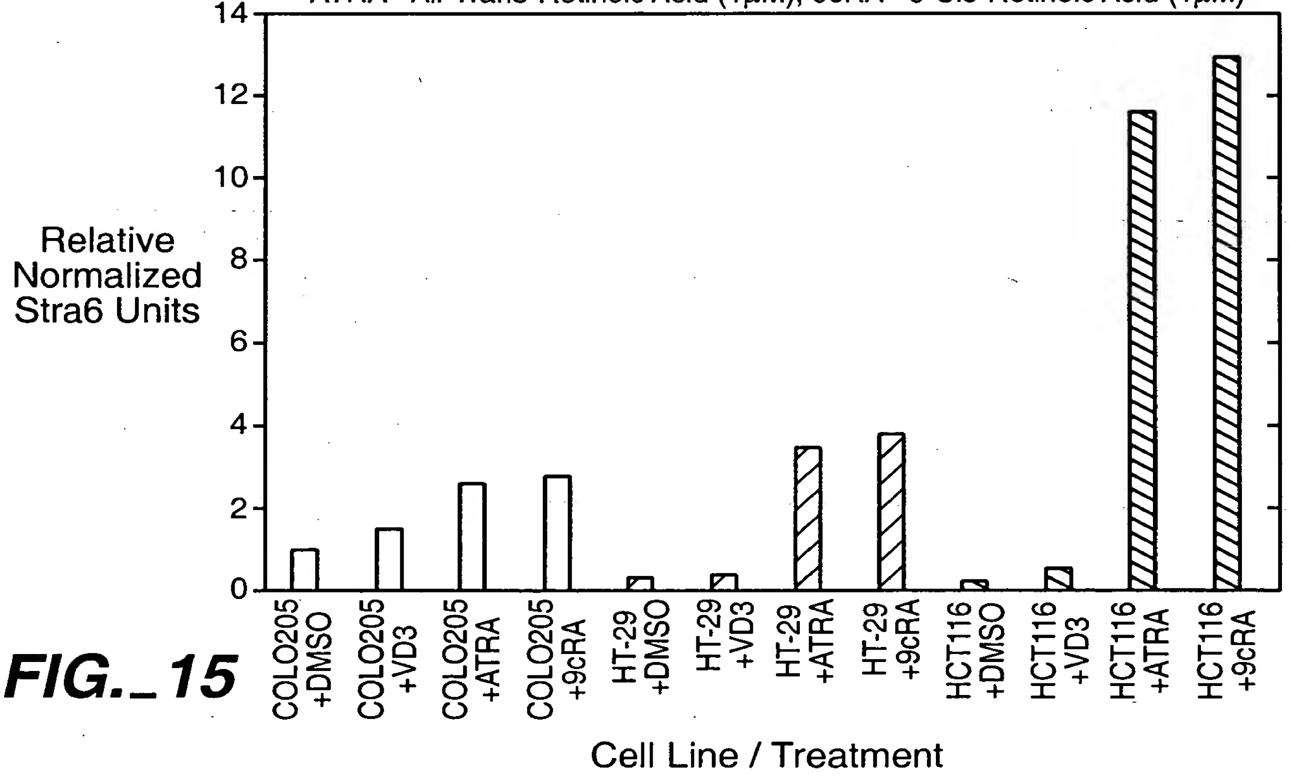
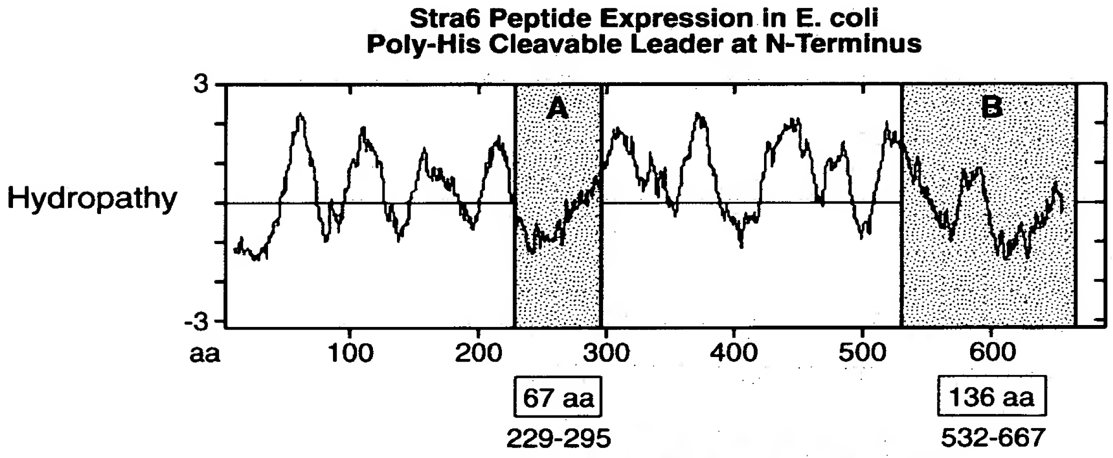


*FIG._15*


Stra6 Peptide Expression in E. coli
Poly-His Cleavable Leader at N-Terminus
Hydropathy
3
-3
aa
100
200
300
400
500
600
A
B
67 aa
229-295
136 aa
532-667

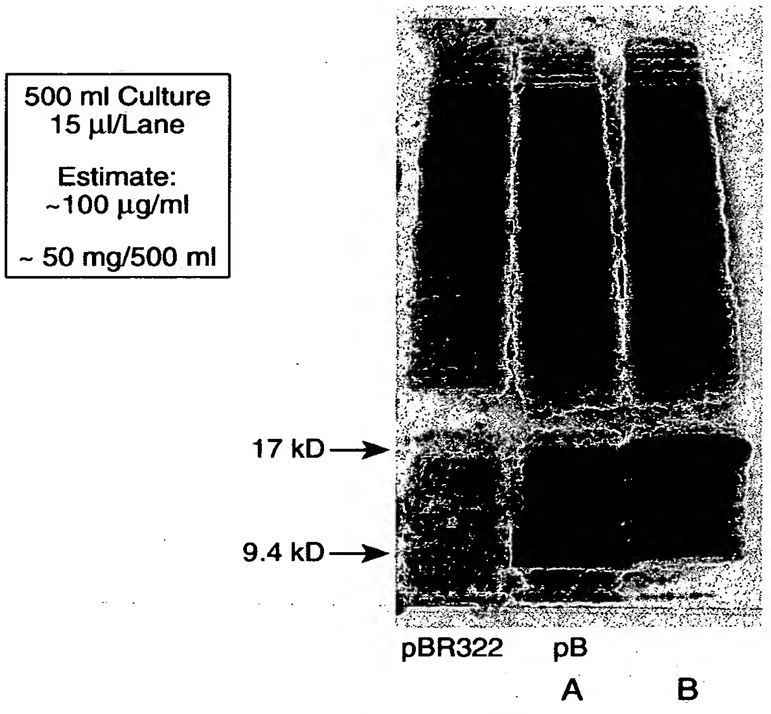

500 ml Culture
15 µl/Lane
Estimate:
~100 µg/ml
~ 50 mg/500 ml
17 kD
9.4 kD
pBR322
pB
A
B

FIG._14

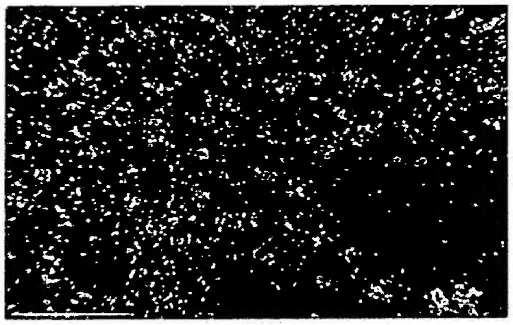


FIG._16A

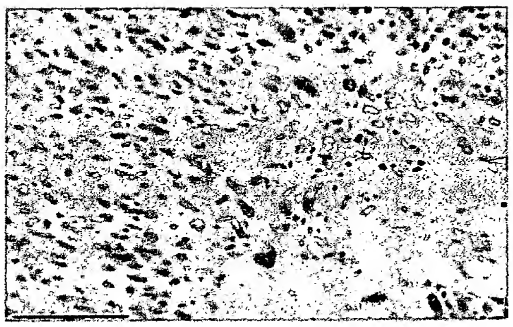


FIG._16B

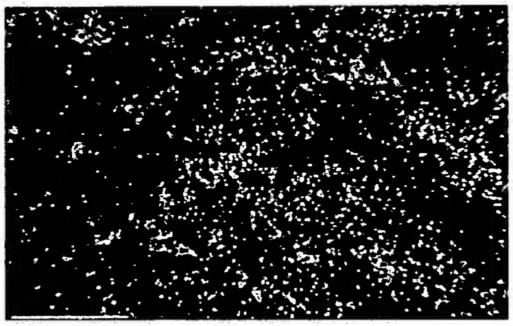


FIG._16C

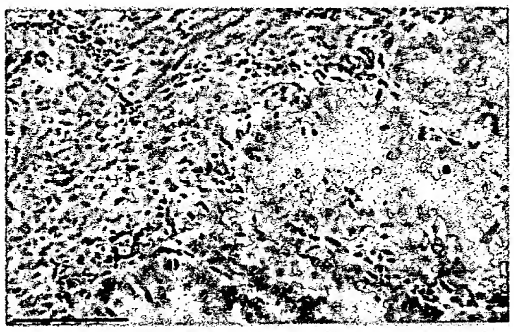


FIG._16D

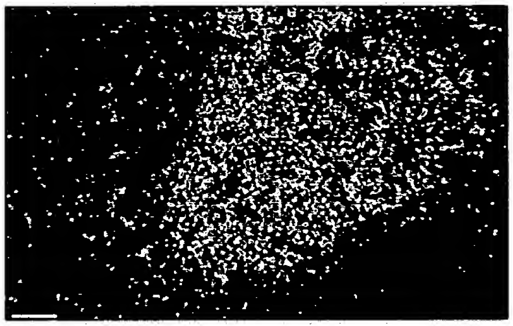


FIG._16E

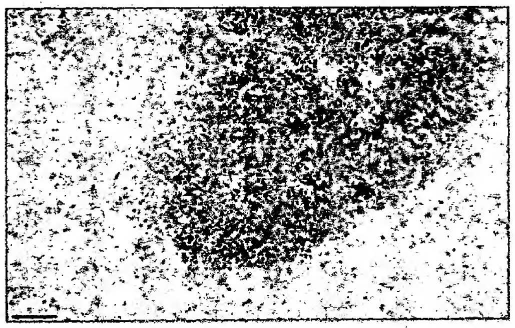


FIG._16F

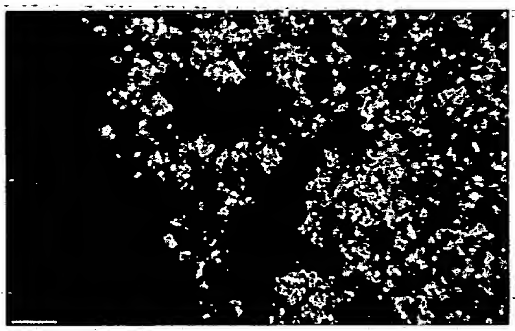


FIG._16G

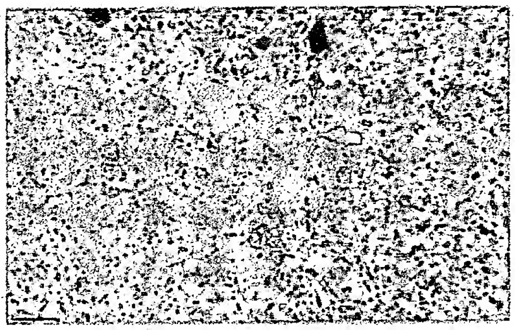


FIG._16H

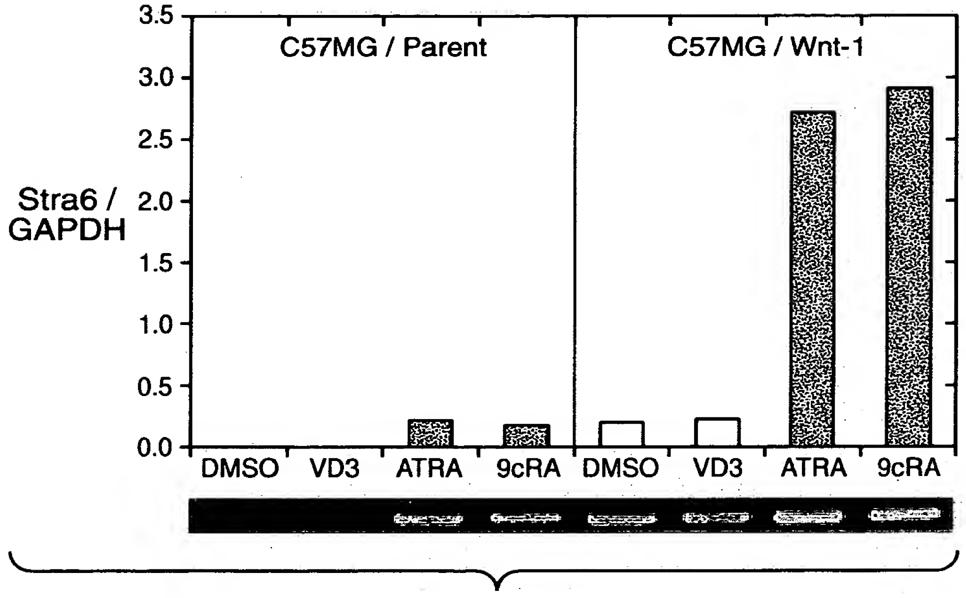


FIG._17A

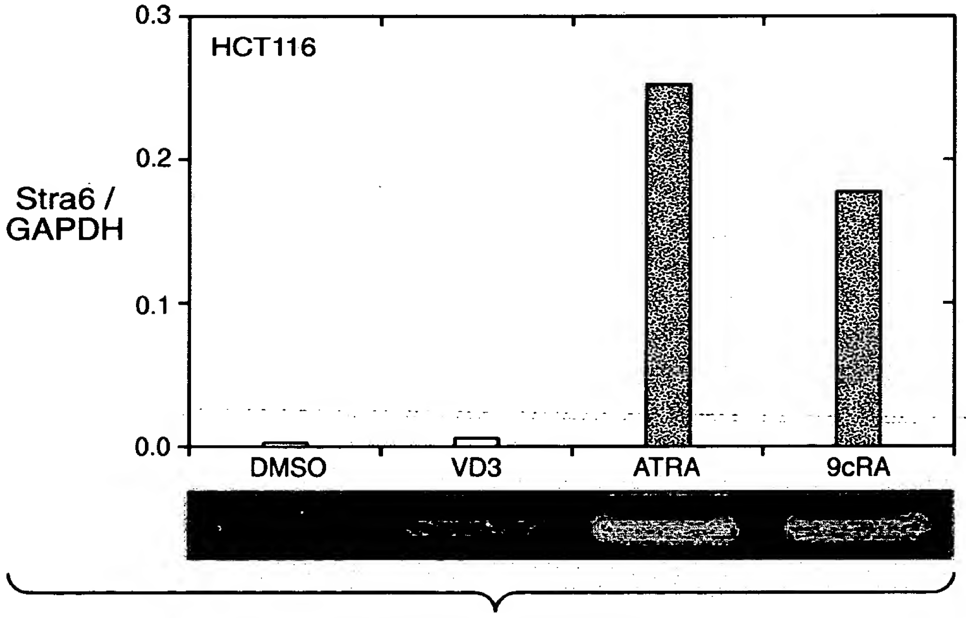


FIG._17C

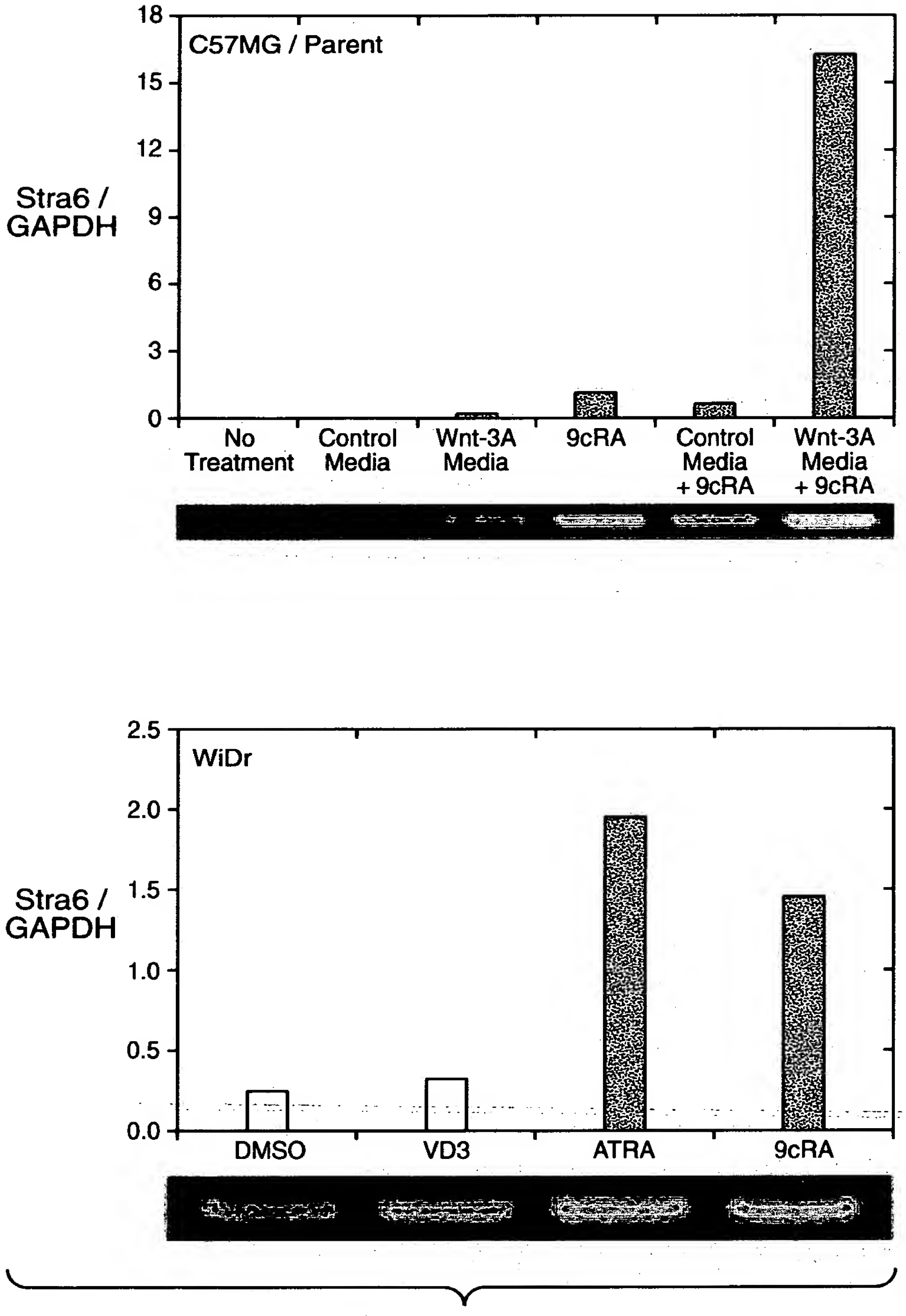

C57MG / Parent
Stra6 / GAPDH
18
15
12
9
6
3
0
No Treatment
Control Media
Wnt-3A Media
9cRA
Control Media + 9cRA
Wnt-3A Media + 9cRA
WiDr
Stra6 / GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
DMSO
VD3
ATRA
9cRA

FIG._17B

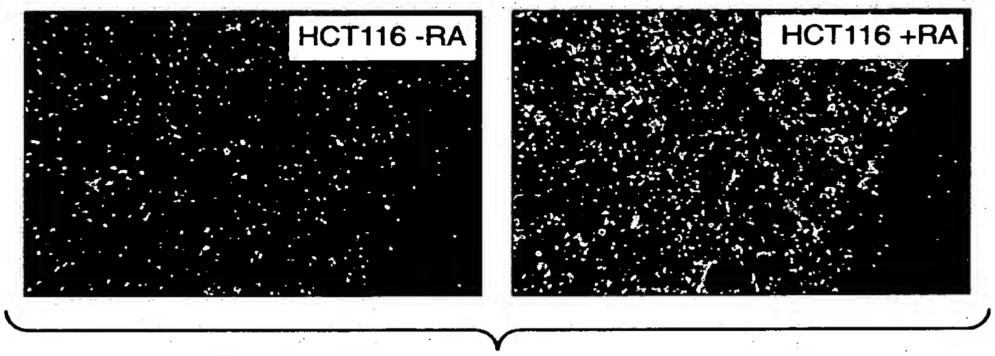


FIG._17D

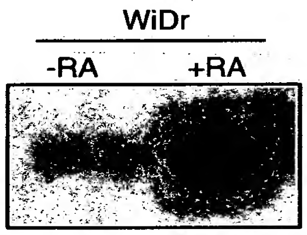


FIG._17E

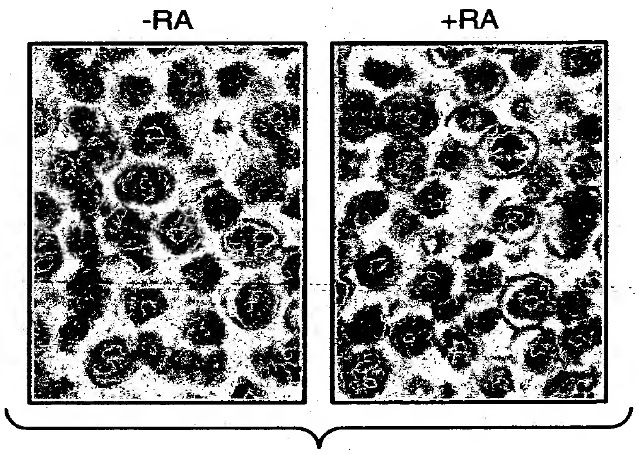


FIG._17F

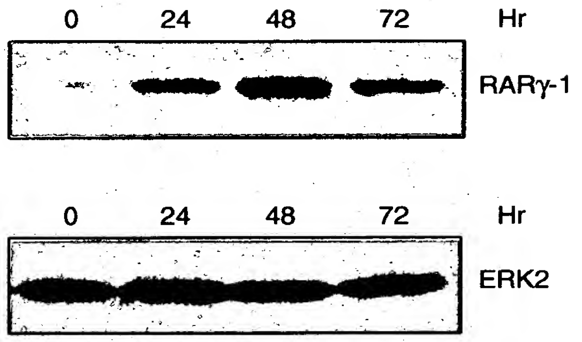


FIG._18A

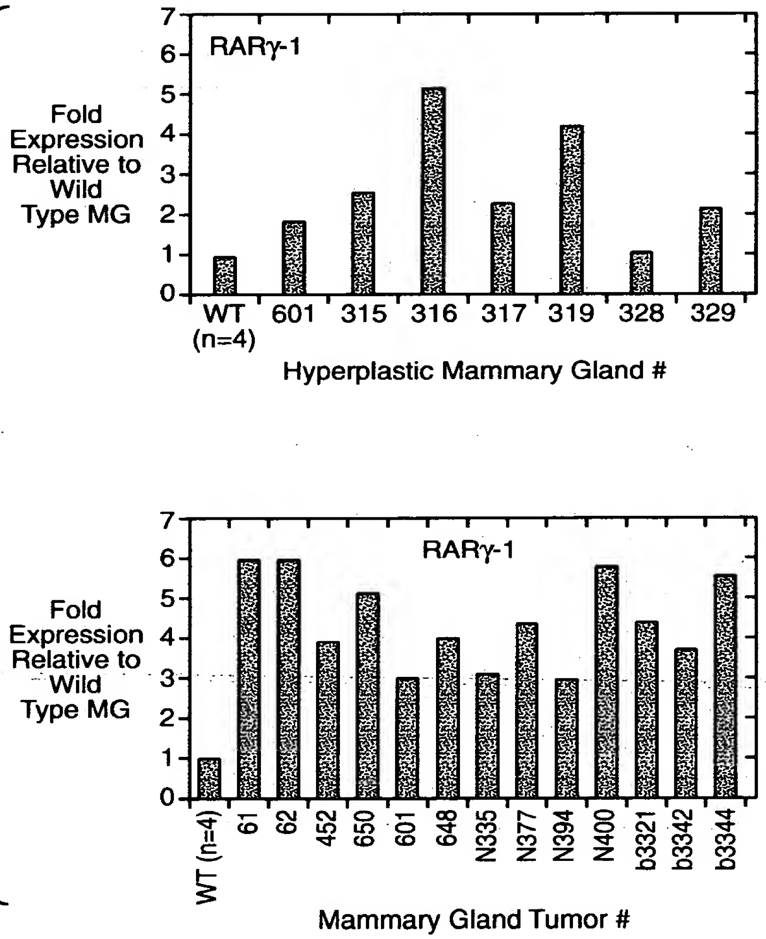


FIG._18B